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Original Communication

Electrolyte analysis of pleural effusion as an indicator of drowning in seawater and freshwater

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A B S T R A C T

It is important for forensic pathologists to determine the diagnosis of drowning as well as the site of drowning. In a previous study, we propose that analysis of electrolytes in pleural effusion from rats may be useful for determining whether drowning has occurred in seawater or freshwater. To test this proposal, we measured the concentration of sodium, potassium and chloride ions and total protein in pleural effusion from 40 autopsy cases: 24 involving seawater drowning, 9 freshwater drowning and 7 no drowning. The concentrations of sodium and chloride ions in pleural effusion showed a significant difference between seawater drowning and freshwater drowning. The concentration of potassium ions and total protein showed no difference between each group, although they increased in proportion to the postmortem interval in cases of both seawater and freshwater drowning. These results are almost same as our previous study and, thus, the quantitative analysis of electrolytes in pleural effusion may be useful for determining whether drowning has occurred in seawater or freshwater.

1. Introduction

Drowning is one of the most frequent causes of accidental death in the world, especially in children.[1] It is important for forensic pathologists to determine the cause of death when victims are recovered from water.[2] If the cause of death is determined as drowning, it is also important to specify where the body had drowned. To obtain clues about the site of drowning, many examinations have been carried out, such as diatom analysis, blood fluoride concentration and the use of immunohistochemical and molecular biological techniques.[3–6] The methodologies used in these studies are useful, however, these require special techniques or instruments.

It is well known that pleural effusion is often present within the thoracic cavities of a drowned body.[2] According to some investigators, pleural effusion more likely occurs if the individual has drowned in seawater rather than in freshwater[5,7,8] and, thus, a comparison of the amount of pleural fluid might be used to determine the site of drowning.

Instead of the volume of pleural effusion, we focused on the difference in the character of the pleural effusion between seawater drowning and freshwater drowning, and found that the concentration of electrolytes in pleural effusion correlated with that in the drowning medium in rat experiments. Also, we proposed the usefulness of analyzing electrolytes in pleural effusion to determine the site of drowning.[9]

The purpose of this prospective study is to examine the usefulness of our simple approach by analyzing concentrations of electrolytes in pleural effusion in our autopsy cases.

2. Subjects and methods

2.1. Samples

Cases of cadavers with over 20 ml pleural effusion in the thoracic cavity were selected together with information on the age, sex, cause of death, postmortem interval and autopsy findings from the forensic autopsies performed at Kyushu University between 2003 and 2007. Of the 44 forensic autopsy cases with over 20 ml pleural effusion, 37 cases were diagnosed as drowning after careful consideration involving several findings, such as detection of diatoms, pulmonary emphysema and edema, Paltauf's spots and froth

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in the air passage. The sites of drowning and postmortem intervals were determined after careful examination of each cases and the time and places where the victims had disappeared and were discovered. Of the 37 drowning cases, two cases were excluded since the postmortem interval was thought to be about 40 days and 3 months, and one case was excluded since the victim had survived after drowning in a bathtub and was treated in hospital for about 2 days. Another case where the victim was thrown into the sea while still alive after having been drowned in a bathtub was also excluded. Therefore, the 40 autopsy cases selected in this study were divided into 3 groups: 24 seawater drowning, 9 freshwater drowning, and 7 no drowning. Of the 33 drowning cases, five cases were unidentified bodies. We estimated the age and the postmortem interval of all five cases by full autopsy examinations and complete police investigations. The postmortem intervals of all 40 cases were under 3 weeks.

Pleural effusion was gathered from the bilateral thoracic cavities of each cadaver. The concentrations of sodium, potassium and chloride ions were measured using an ion-selective electrode and the concentration of total protein was measured using Biuret's method.

2.2. Statistical analyses

All statistical analyses were performed using JMP®version 6, Japanese Edition (SAS Institute, INC., Cary, NC, USA). The difference between the above three groups was calculated by Tukey–Kramer's procedure as a post-hoc test. Linear regression analysis was carried out in three drowning groups (24 seawater drowning, 9 freshwater drowning, and 33 total drowning) to determine the relationship between the concentration of electrolytes or total protein in pleural effusion and the postmortem interval. A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Electrolyte analysis of pleural effusion

Table 1 shows the concentrations of electrolytes and total protein in pleural effusion, and the postmortem interval in 33 drowning cases. Table 2 shows the concentrations of electrolytes and total protein in pleural effusion, the postmortem interval and cause

Table 1
Concentrations of electrolytes in pleural effusion from autopsy cases of individuals who had drowned.

Case no.	Age	Sex	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	TP (g/dl)	Postmortem interval (days)	Drowning location
1	8	F	147	39.4	141	n.a.	1.25	Seawater
2	28	M	166	47.5	183	n.a.	4.5	
3	53	M	102	60.4	90	n.a.	7	
4	Un(60–69)	F	127	11.1	89	5.3	3.5	
5	38	M	128	42.35	104.5	5.55	2.25	
6	18	M	170	60.9	182	n.a.	14	
7	29	M	147.5	62.25	146.5	13.2	21	
8	46	M	189	50.25	198.5	7.95	14	
9	68	F	150	25.15	132.5	2.75	1	
10	Un(40–49)	M	147	39	147	5.8	6	
11	43	M	150.5	47.15	153.5	8.8	3	
12	50	M	154	33.9	170.5	5.4	1.75	
13	60	M	284	20.6	315	0.9	3.5	
14	63	F	161.5	27.35	150	2.2	3.5	
15	65	M	154	24.2	146.5	1.6	0.83	
16	64	F	178	29.8	174	3.2	3.75	
17	70	M	243.5	17.5	265	1.35	2.25	
18	57	M	175.5	47.85	181.5	5.05	8.5	
19	Un(60–69)	F	191.5	22.8	182.5	2.55	2.25	
20	66	M	117	45.1	116	8.8	6	
21	Un(50–59)	M	333	34.35	387	5.55	5	
22	62	F	145.5	30.55	113.5	3.6	1.75	
23	Un(50–59)	M	211	59.15	231.5	7.55	17.5	
24	61	M	220	45.2	292.5	5	21	
25	48	M	99	38.95	72.5	5.65	2.5	Freshwater
26	79	M	82	50.8	64	9.15	6	
27	52	F	96	73	32	5.4	1.2	
28	25	F	75	52.05	62.5	7.4	7	
29	54	M	78.5	20.75	60	4.1	1.25	
30	60	M	70	54.35	61	9.2	8	
31	40	M	49.5	33.7	49.5	6.75	0.75	
32	75	M	67	40.1	61	6.6	6	
33	23	F	61	26.2	47.5	5.8	2.25	

Un: unknown (estimated age), M: male, F: female, n.a.: not analyzed.

Table 2
Concentrations of electrolytes in pleural effusion and causes of death of autopsy cases of individuals who had not drowned.

Case no.	Age	Sex	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	TP (g/dl)	Postmortem interval (days)	Cause of death
34	19	F	92	62.6	70	n.a.	4.5	Phenobarbital intoxication
35	59	M	130	17.5	110	3	0.4	Hypoxic ischemic encephalopathy
36	47	M	165.5	17	124	1.95	0.1	Alcoholic myocardiopathy
37	41	M	88	72.5	71	8.2	8	Intracranial injury
38	85	F	141	12.9	109	4.7	0.7	Paralytic ileus
39	55	F	133	11.1	106	2	0.66	Bronchopneumonia
40	78	M	132.5	24.75	107.5	2.6	3	Lobar pneumonia

M: male, F: female.

Table 3
Concentrations of electrolytes in pleural effusion in the three groups.

	n	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	TP (g/dl)	Postmortem interval (days)	Age (years)
Seawater	24	174.7 ± 53.0	38.5 ± 14.6	178.9 ± 73.2	5.1 ± 3.1	6.5 ± 6.3	49.9 ± 18.1
Freshwater	9	75.3 ± 15.9*	43.3 ± 16.0	56.7 ± 11.9*	6.7 ± 1.7	3.9 ± 2.8	50.7 ± 19.5
Not drowning	7	126.0 ± 27.4*	31.2 ± 25.4	99.6 ± 20.8*	3.7 ± 2.4	2.5 ± 2.9	54.9 ± 22.4

Na^+, sodium ions; K^+, potassium ions, Cl^-, chloride ions; TP, total protein ($n = 20$ in seawater drowning, $n = 6$ in no drowning), age ($n = 19$ in seawater drowning). Values are means ± SD. Significantly different from seawater.
* $P < 0.05$.

of death in seven cases not involving drowning. Significant differences were observed in the concentration of sodium and chloride ions in pleural effusion between seawater and the other two groups, freshwater and no drowning. The concentration of potassium ions and total protein in pleural effusion showed no significant differences between these three groups (Table 3).

3.2. Relationship between the postmortem interval and concentration of electrolytes in pleural effusion

The concentration of sodium and chloride ions in pleural effusion did not correlate with the postmortem interval in any of the seawater (Na^+: adjusted R-square = −0.0301, Cl^-: adjusted R-square = 0.0175), freshwater (Na^+: adjusted R-square = −0.1398, Cl^-: adjusted R-square = 0.1392), and total drowning groups (Na^+: adjusted R-square = 0.0198, Cl^-: adjusted R-square = 0.0731) (Fig 1).

The concentration of potassium ions and total protein in pleural effusion significantly increased in proportion to the postmortem interval in all 3 groups except for the concentration of potassium ions in pleural effusion in the freshwater group (seawater K^+: adjusted R-square = 0.4531, seawater TP: adjusted R-square = 0.3549, freshwater K^+: adjusted R-square = −0.0072, freshwater TP: adjusted R-square = 0.5752, total drowning K^+: adjusted R-square = 0.2893, total drowning TP: adjusted R-square = 0.2717) (Fig. 2a and b).

4. Discussion

In our previous study using rats we described that the concentration of electrolytes in pleural effusion was correlated with the concentration of electrolytes in the drowning water and with the duration of the postmortem interval.[9]

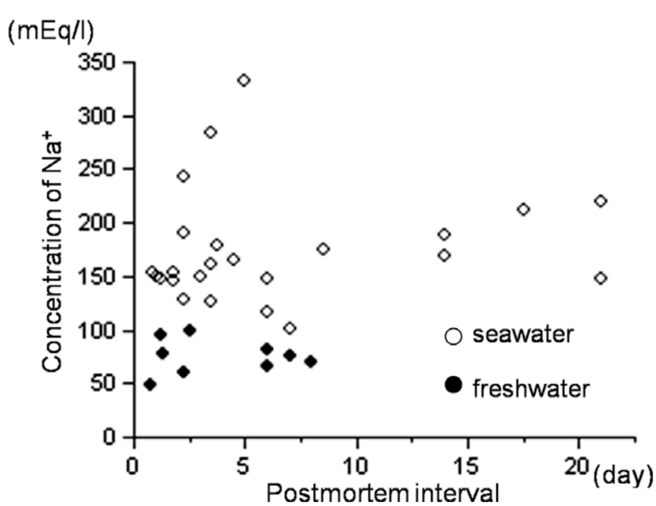

Fig. 1. Concentrations of sodium ions of drowning cases.

In this prospective study of our autopsy cases, the average concentration of sodium and chloride ions in pleural effusion in seawater drowning was significantly higher than that in freshwater drowning (Table 3). As shown in Table 1, the concentrations of sodium and chloride ions in pleural effusion in all freshwater drowning cases were less than 100 mEq/l. In contrast to this, the concentrations of sodium and chloride ions in all seawater drown-

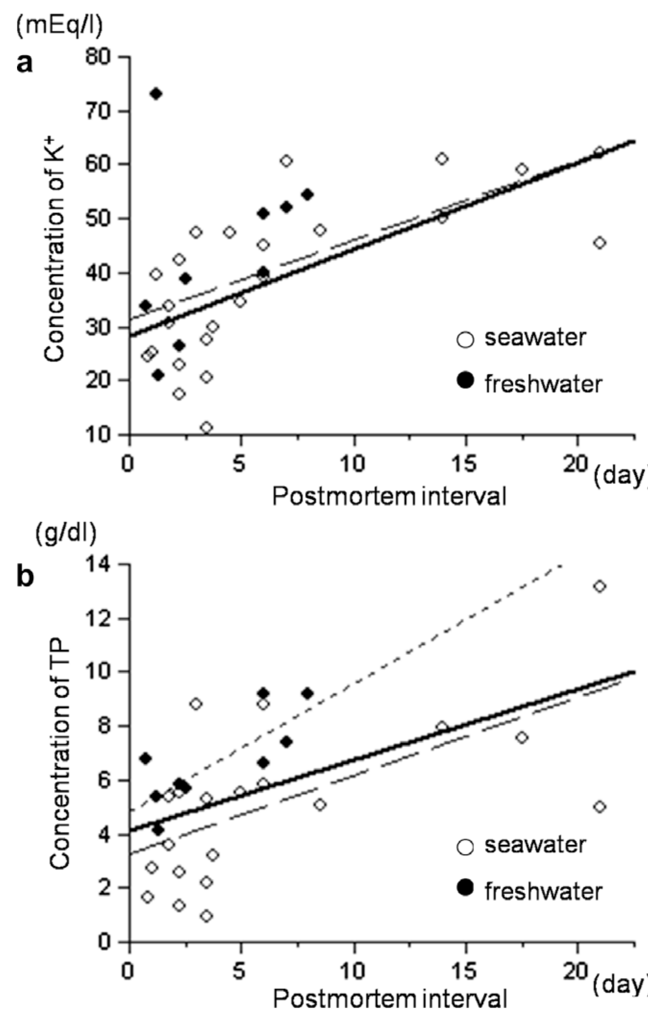

Fig. 2. Regression lines of potassium ions (a) and total protein (b) of drowning cases. Dashed, dotted and solid lines show the relationship between the postmortem interval and the concentration of potassium ions or total protein in pleural effusion of seawater drowning, freshwater drowning and total drowning group, respectively. K^+ (a) Seawater drowning: $Y_K = 28.09X_{day} + 1.610$ ($n = 24$, adjusted R-square = 0.4531, $P = 0.0002$), Total drowning: $Y_K = 31.27X_{day} + 1.482$ ($n = 33$, adjusted R-square = 0.2893, $P = 0.0007$) TP (b) Seawater drowning: $Y_{TP} = 3.232X_{day} + 0.2915$ ($n = 20$, adjusted R-square = 0.3549, $P = 0.0033$), Freshwater drowning: $Y_{TP} = 4.822X_{day} + 0.4761$ ($n = 9$, adjusted R-square = 0.5752, $P = 0.0108$), Total drowning: $Y_{TP} = 4.098X_{day} + 0.2648$ ($n = 29$, adjusted R-square = 0.2717, $P = 0.0022$) K^+, potassium ions; TP, total protein.

ing cases were more than 100 mEq/l with the exception of chloride ions in Case 3 (90 mEq/l) and Case 4 (89 mEq/l). As shown in Table 3, the postmortem interval showed variation between seawater, freshwater and not drowning groups, however, this variation was not significant from the statistical point of view. Although the different interval time might alter the results of the electrolytes analysis, the concentrations of sodium and chloride ions in seawater and freshwater drowning cases had significant differences in the entire interval of our cases (Fig. 1).

Although the mechanism of formation of pleural effusion is not fully understood, these differences in electrolytes might be induced by osmotic pressure differences and the amount of aspirated immersion medium. In seawater drowning, hyperosmolar water containing high concentrations of sodium and chloride ions probably leads to plasma leaking out of the capillaries present in the lung interstitials, whereas in freshwater drowning, hypoosmolar water with low concentrations of sodium and chloride ions leaks into the capillaries in the lung interstitials which causes hemodilution. Since the pleural membranes are thin and exhibit little resistance to the movement of liquid and protein, as shown for the peritoneal mesothelium,[10] the lung interstitial liquid may cross leaky pleural membranes easily and move into the pleural space.[11] These mechanisms are in agreement with the Byard et al. suggestion that the sodium levels in left ventricular blood could be used as an indicator of hemodilution in freshwater drowning.[12]

The effect of pleural effusion present in the cadaver before drowning is not known. However, we observed significant differences in the concentrations of sodium and chloride ions between seawater drowning and no drowning groups, and the concentrations of sodium and chloride ions were under 165.5 and 124 mEq/l, respectively, in no drowning group. Therefore, if the concentrations of sodium and chloride ions in pleural effusion of the cadaver are higher than 170 mEq/l and 125 mEq/l, respectively, this is a strong indication of seawater drowning.

Yorulmaz et al.[7] and Zhu et al.[5] reported that the total amount of pleural effusion in seawater drowning was greater than that in freshwater drowning. Although similar results were obtained in our study (data not shown), it was considered that the volume of pleural effusion could be influenced by the amount of pleural effusion present before drowning. Therefore, our approach using electrolyte concentrations in pleural effusion provides more reliable evidence for determining the site of drowning.

The average concentrations of potassium ions and total protein in pleural effusion were not significantly different between seawater drowning and freshwater drowning. However, as shown in Fig. 2a and b, there was the correlation between the postmortem interval and the concentration of potassium ions and total protein in pleural effusion in seawater drowning and total drowning. In freshwater drowning, the concentration of potassium ions in pleural effusion did not correlate with the postmortem interval, because in Case 27, the concentration of potassium ions was extremely high (73 mEq/l). This high concentration of potassium ions in Case 27 may be derived from the original pleural effusion before drowning. The concentration of total protein in pleural effusion in freshwater drowning was also correlated with the postmortem interval. Accordingly, the concentration of potassium ions and the total protein may be useful for estimating the postmortem interval in drowning cases.

In conclusion, our results obtained by the analyses of electrolytes in autopsy cases were almost the same as those in our previous animal study.[9] Further investigations on the detailed mechanism of the formation of pleural effusion in drowning cases and the effects of the original pleural effusion are needed. However, the quantitative analysis of electrolytes in pleural effusion is useful for determining whether drowning has occurred in seawater or freshwater. The concentrations of potassium ions and total protein in pleural effusion can be used for rough estimation of the postmortem interval in drowning cases.

Conflict of interest statement

None declared.

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Ethical approval

None declared.

References

1. Frederic S, Jean LB. Drowning: a review of epidemiology, pathophysiology, treatment and prevention. *Resuscitation* 2004;**63**:261–8.
2. Michel HAP, Els ADL. Drowning: still a difficult autopsy diagnosis. *Forensic Sci Int* 2006;**163**:1–9.
3. Ludes B, Coste M, North N, Doray S, Tracqui A, Kintz P. Diatom analysis in victim's tissues as and indicator of the site of drowning. *Int J Legal Med* 1999;**112**:163–6.
4. Chen YC, Deng ZK, Zhu JZ. The significance of detecting serum fluorine level in the diagnosis of drowning. *Forensic Sci Int* 1990;**46**:289–94.
5. Zhu BL, Ishida K, Quan L, et al. Pulmonary immunohistochemistry and serum levels of a surfactant-associated protein A in fatal drowning. *Legal Med* 2002;**4**:1–6.
6. Zhu BL, Ishida K, Taniguchi M, et al. Possible postmortem serum markers for differentiation between fresh-, saltwater drowning and acute cardiac death: a preliminary investigation. *Legal Med* 2003;**5**:S298–301.
7. Yorulmaz C, Arican N, Afacan I, Dokgoz H, Asirdizer M. Pleural effusion in bodies recovered from water. *Forensic Sci Int* 2003;**136**:16–21.
8. Zhu BL, Quan L, Li DR, et al. Postmortem lung weight in drownings: a comparison with acute asphyxiation and cardiac death. *Legal Med* 2003;**5**:20–6.
9. Inoue H, Ishida T, Tsuji A, Kudo K, Ikeda N. Electrolyte analysis in pleural effusion as an indicator of the drowning medium. *Legal Med* 2005;**7**:96–102.
10. UpToDate®. Mechanisms of pleural liquid turnover in the normal state. <http://www.utdol.com/online/content/topic.do?topicKey=pleurdis/5304&selectedTitle=14~150&source=search_result>; 2005 [retrieved 30.04.08].
11. UpToDate®. Mechanisms of pleural liquid accumulation in diasease. <http://www.utdol.com/online/content/topic.do?topicKey=pleurdis/7469&selectedTitle=13~150&source=search_result>; 2005 [retrieved 30.04.08].
12. Byard RW, Cains G, Simpson E, Eitzen D, Tsokos M. Drowning, haemodilution, haemolysis and staining of the intima of the aortic root – preliminary observations. *J Clin Forensic Med* 2006;**13**:121–4.